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Attachment of gonococci to sperm
Influence of physical and chemical factors

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Summary

Neisseria gonorrhoeae of pilated T1 and nonpilated T4 colony types attached themselves to human sperm in greatest numbers and most reproducibly when suspensions of the cells were incubated at 35° C in Ringer's solution, pH 6·8. After incubation for 15 or 30 min. in a water bath shaker, 50 per cent. of human sperm had T1 gonococci attached and 25 per cent. T4.

Sperm and both types of gonococci were pre-incubated separately with various chemical agents, selected because the agent is found in genital fluids, or has a known effect on bacterial cell walls or sperm membrane. After treatment, sperm were washed or were not washed, and were then tested for attachment by mixture with untreated gonococci. Treated gonococci were handled in the same manner. Change in the percentage of attachment was defined as deviation from the range expected on the basis of a standard curve. Treatment of sperm with the nucleotides, ATP or cAMP, curtailed attachment by T1 gonococci but had no effect on attachment by T4. Treatment of gonococci with calcium salts and the enzymes, trypsin, alpha chymotrypsin, and lysozyme, reduced attachment of T1 bacteria to the percentage expected for nonpilated T4 gonococci. Electron micrographs of T1 gonococci treated with concentrations of enzymes sufficient to reduce attachment showed loss of pili. Attachment of both T1 and T4 gonococci was enhanced by treatment of bacteria with iron salts. These data demonstrated that attachment is not a simple sticking together of eukaryotic and prokaryotic cells. Rather, there are different sites on the surfaces of sperm and of gonococci that facilitate attachment. The sites are not the same for both cell types because every effective agent affected either sperm or bacteria, but never both.

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Introduction

Attachment of *Neisseria gonorrhoeae* to cells of the prospective human host is probably the initial step of infection (Swanson, 1973; Ward and Watt, 1972; 1973). We reported that pilated, virulent gonococci of colony types 1 (T1) and 2 (T2) (Jephcott, Reyn, and Birch-Andersen, 1971; Kellogg, Peacock, Deacon Brown, and Pirkle, 1963; Swanson, Kraus, and Gotschlich, 1971) attached to a greater percentage of sperm than did nonpilated, avirulent cells of colony type 4 (T4) (James-Holmquest, Swanson, Buchanan, Wende, and Williams, 1974). Most eukaryotic cells have some phagocytic capability but human sperm do not. Thus, attachment *per se* between gonococci and sperm can be examined without concurrent or subsequent phagocytosis.

In the present study, the effect of certain chemical and biochemical agents on attachment was determined. Agents were selected that are found in the male or female reprodutive tracts, or that have specific defined effects on bacterial cell walls or sperm membranes. Each component of the system, sperm, T1, and T4 gonococci, was tested separately to determine whether a given agent did or did not affect attachment.

Material and methods

Growth and preparation of bacteria

Two colonial types, T1 and T4, isolated from *N. gonorrhoeae* strain WP by the method of Kellogg and others (1963) were maintained by transfer on GCB medium (GC Agar Base Medium plus 1 per cent. IsoVitaleX, Baltimore Biological Laboratory, Cockeysville, Md.). Bacteria of types T1, T4, and T4* (Swanson, Sparks, Zeligs, Siam, and Parrott, 1974) isolated from *N. gonorrhoeae* strain MS11 were kept in the same manner.

Examination of negatively stained preparations by electron microscopy confirmed the presence or absence of pili from T1, T4, and T4* gonococci.

After incubation of cultures in candle-extinction jars at 35°C for 18 to 20 hrs, gonococci were removed from the agar surface with a cotton swab previously moistened in Ringer's solution (RS) composed of NaCl, 8·5 g.; KCl, 0·3 g.; $CaCl_2$. $2H_2O$, 0·33 g.; per litre of distilled, deionized water (Diem and Lentner, 1970). After treatment by the method of Swanson and others (1974) to remove all clumps of gonococci from the suspension in RS, the concentration of gonococci per ml. was determined (James-Holmquest and others, 1974) by counting in a Petroff-Hausser chamber (Hausser Scientific, Blue Bell, Pennsylvania).

We present data from experiments in which sperm or gonococci of each colony type were incubated before mixture for each agent studied because the addition of agents at the time of mixing sperm and gonococci did not affect attachment except in the case of iron salts. This period of incubation is termed pre-incubation to distinguish this step from the incubation time for analysis of attachment. For pre-incubation of gonococci, the agent was added to suspensions of bacteria in RS, and the mixtures were incubated at 35°C for the appropriate time in a water bath shaker (Gyrotory Water Bath Shaker, New Brunswick Scientific Co., Inc. New Brunswick, N.J.) set at 145 r.p.m. Concentrations of bacteria were determined either immediately after pre-incubation, or, for certain experiments, after gonococci were washed twice by centrifugation. After each of these centrifugations (Sorvall RC2-B, Ivan Sorvall, Inc., Norwalk, Conn.) at 19,000 G for 15 min., the supernatant fluid was decanted, and the bacteria in the pellet were re-suspended in RS. The concentration of gonococci for both washed and unwashed specimens was determined as previously described (James-Holmquest and others, 1974), and RS was added to obtain suspensions containing 10^7 to 10^8 bacteria/ml.

Preparation of sperm

The J. Sayles Leach Urological Research Laboratory, Baylor College of Medicine, Houston, Texas, provided samples of human semen with normal sperm counts. Criteria for acceptability of specimens and the procedure for removing seminal fluid, except for the use of RS instead of M199 as suspension medium, remained the same as those previously described (James-Holmquest and others, 1974). Pooled and diluted specimens of sperm contained 1×10^9 cells/ml. The total suspension was divided into 2 ml. amounts in polystyrene Snap-Cap, 12×75 mm. tubes (DiSPo culture tubes, Scientific Products, Evanston, Ill.). After the addition of glycerol (10 per cent. v/v) to the tubes, each tube was mixed by agitation on a Vortex Jr. mixer (Scientific Industries, Inc., Greens Village, N.Y.). Finally, these preparations were frozen in liquid nitrogen and stored at —70°C until needed.

Specimens for use were thawed rapidly in a water bath at 36°C, were centrifuged at 1500 G in a clinical centrifuge (Model CL, International Equipment Co., Needham Heights, Mass.) for 5 min. and were then re-suspended in fresh RS after the supernatant fluid was decanted. After two such washings, the sperm suspension contained 10^7 to 10^8 cells/ml., as determined by counts with a haemocytometer.

Pre-incubation of sperm with each agent was done as described for bacteria except for the centrifugation procedure. When washed after pre-incubation, sperm were centrifuged twice at 1500 G for 5 min. and re-suspended in fresh RS each time.

Mixture of sperm with bacteria

The effect of each chemical and biochemical agent was determined separately for each component in the system: sperm, *N. gonorrhoeae* T1, or T4. Sperm pre-incubated with each agent were mixed with untreated T1 or T4 gonococci. Both types of gonococci were pre-incubated separately with each agent, and then these bacterial suspensions were mixed with untreated sperm. Samples (0·5 ml.) of sperm (10^7 to 10^8 cells/ml.) were mixed with an equal volume of bacteria (10^7 to 10^8 cells/ml.) in the polystyrene tubes and incubated at 35°C in the water bath shaker for periods lasting up to 60 min.

Controls

Untreated sperm and gonococci of both types were mixed and incubated immediately after their respective concentrations were established. If the expected percentage of attachment did not occur with these specimens, the experiment was discarded. In addition, to determine if the solvents in which the agents were suspended, or the volume of the solvent, affected the percentage of attachment, equal proportions of the solvents alone were added to sperm or bacteria. These specimens were pre-incubated, mixed, incubated, and counted exactly like the test specimens. In no case did the solvent or volume alter the percentage of attachment.

Evaluation of attachment of bacteria to sperm

At various times, three separate drops of the mixture of bacteria and sperm were pipetted on to a clean glass slide and were allowed to dry in air. The effect of variation in time required for slides to dry and in percentage of attachment that could occur during these times was randomized by use of twenty experiments to define expected range in percentage of attachment. The slides were fixed in 95 per cent. (v/v) aqueous ethanol for 5 min., rinsed with water, flooded with crystal violet (modified Hucker's crystal violet, Gram 1, Paik, 1970) for 15 min., washed with water, and then blotted dry.

A minimum of 100 sperm for each of the 3 drops per slide was counted by light microscopy at a magnification of 400×. The number of counted sperm with bacteria attached, divided by the total number of sperm counted, multiplied by 100, yielded the percentage of attachment. The mean and standard deviation were claculated from the values of percentage of attachment for three to twenty samples, each slide of 3 drops representing one sample.

Salts

Three iron compounds, ferrous sulphate ($FeSO_4 \cdot 7H_2O$: J. T. Baker Chemical Company, Phillipsburg, N.J.), ferric nitrate ($Fe(NO_3)_3 \cdot 9\ H_2O$: Fisher Scientific Co., Fairlawn, N.J.), and ferric chloride ($FeCl_3 \cdot 6\ H_2O$: J. T. Baker Chemical Company), each diluted in water to 100 mM, comprised the stock solutions. These solutions

were sterilized by filtration (0·20 μm Nalgene membrane, filter unit, Nalge-Sybron Corp., Rochester, N.Y.), stored at 4°C in light-proof containers, and diluted at the time of use to the desired concentration. In a similar fashion, stock solutions of magnesium chloride ($MgCl \cdot 6H_2O$: J. T. Baker Chemical Co.) and cupric sulphate ($CuSO_4 \cdot 5H_2O$: J. T. Baker Chemical Co.) were prepared. Determination of the effect of calcium salt ($CaCl_2 \cdot 2H_2O$: J. T. Baker Chemical Co.) required use of three different solutions of RS: one with no $CaCl_2$, one with 2·2 mM $CaCl_2$, and a third with 22 mM $CaCl_2$.

Nucleotides

Adenosine 3′ :5′ -cyclic monphosphoric acid sodium salt (cAMP, Sigma Chemical Co.) and adnosine triphosphate crystalline disodium (ATP, Nutritional Biochemicals Corp., Cleveland, Ohio) were prepared in stock solutions of 0·15 mM, pH 6·8, and stored at 0°C in 1 ml. amounts in glass vials. A fresh vial was used for each experiment.

Enzymes

Trypsin (E. C. No. 3. 4. 4. 4) of type 111 from bovine pancreas (Sigma Chemical Co.) and alpha chymotrypsin (E. C. No. 3. 4. 4. 5) from bovine pancreas (Calbiochem, LaJolla, California) were prepared in stock solutions of 10 mg./ml. by dilution of the lyophilized enzymes in water. Glass vials containing 1 ml. of solution were stored at 0°C, and a fresh vial was used for each experiment.

Lysozyme (muramidase, E. C. No. 3. 2. 1. 17) obtained from egg white (Sigma Chemical Co.), compounded as a stock solution of 100 mg./ml. and stored at 4°C, was freshly prepared each week. The Influenza Research Center, Baylor College of Medicine, Houston, Texas, furnished neuraminidase (E. C. No. 3. 2. 1. 18) as receptor-destroying enzyme (RDE, *Vibrio comma* extract, Microbiological Associates, Rockville, Md.). The enzyme was stored at 0°C. For experiments, solutions were thawed and used without dilution.

Results

Standard curve

Physical circumstances, such as the type of suspension medium, pH, and temperature, were examined to define optimal conditions for the system. When incubated in RS, the percentage of sperm with bacteria attached was identical to that obtained with M199 (James-Holmquest and others, 1974). The simpler composition of RS was an advantage in evaluating the effect of agents added to suspensions. A pH of 6·8 and temperature of 35°C were selected as optimum after determination of percentage of attachment at temperatures from 5 to 40°C in RS solutions at a range of pH 5 to 8. Experiments done with these optimal conditions provided data for a standard curve (Fig. 1) that is a reference for the expected percentage of attachment at specific times over a period of one hour.

Statistically significant differences between attachment for T1 and T4 bacteria were observed after incubation for 1, 15, and 30 min., and these times

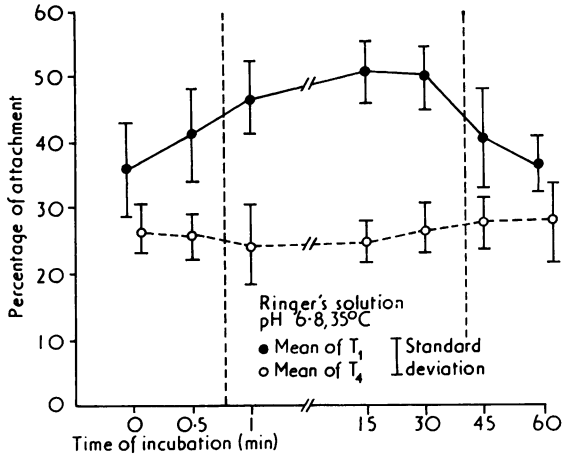

FIG. 1 *Standard curves obtained when T1 or T4* Neisseria gonorrhoeae *were mixed with sperm and incubated in a water bath shaker under the conditions indicated in a water bath. Area between the dotted lines includes the times at which the difference between T1 and T4 gonococci in percentage of attachment to sperm is statistically significant. These data are the result of twenty different experiments.*

were used for all other experiments. Identical results were obtained with WP T1 or MS11 T1, and with WP T4, or MS11 or T4*. We found no difference between T4 and T4* in our system. In evaluating the effect of various agents on attachment, significant change is defined as a mean, and in most cases a range, in percentage of attachment above or below that of the standard curve for samples taken at the same times.

Effect of salts

Of the four salts tested, iron, calcium, magnesium, and copper, only two affected attachment, and these acted solely on the bacteria. Iron salts in either the ferrous or ferric forms significantly enhanced the ability of gonococci to attach to sperm (Fig. 2A, B, and C). Pilated T1 bacteria were affected to a greater degree than nonpilated gonococci at greater concentrations of $Fe(NO_3)_3$ and $Fe\ SO_4$. After the iron salts were diluted to concentrations of 0·001 mM, no effect on attachment was apparent. The effect on attachment was neither enhanced nor removed if the bacteria were washed before mixture with sperm. No increase in pilation was detected by electron microscopy of bacteria treated with iron. Calcium as a chloride salt significantly depressed attachment for T1 bacteria at a concentration of 22 mM (Fig. 2D), a greater concentration than the 4 mM reported to be present in uterine fluid (Bishop, 1961) or the 6 mM reported for semen (Diem and

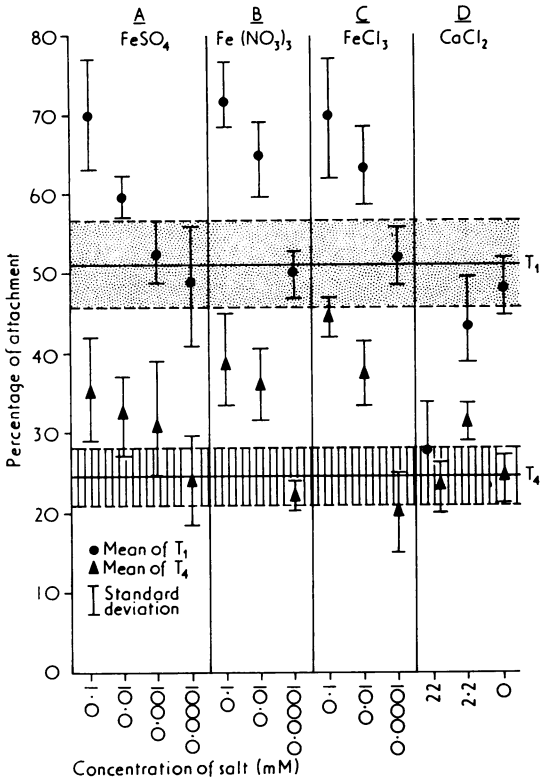

FIG. 2 *Effect of various concentrations of iron (A, B, and C) and calcium (D) salts on ability of T1 and T4 gonococci to attach to untreated sperm. Gonococci were pre-incubated with the salts for* 30 *min. at* 35°*C before mixture with sperm. After the mixture had been incubated for an additional* 15 *min. in a water bath shaker at* 35°*C, the percentage of sperm with gonococci attached was determined. Stippled and lined areas show expected range for percentage of attachment of T1 and T4 gonococci respectively, when bacteria and sperm were incubated under standard conditions for* 15 *min. (see Fig.* 1).

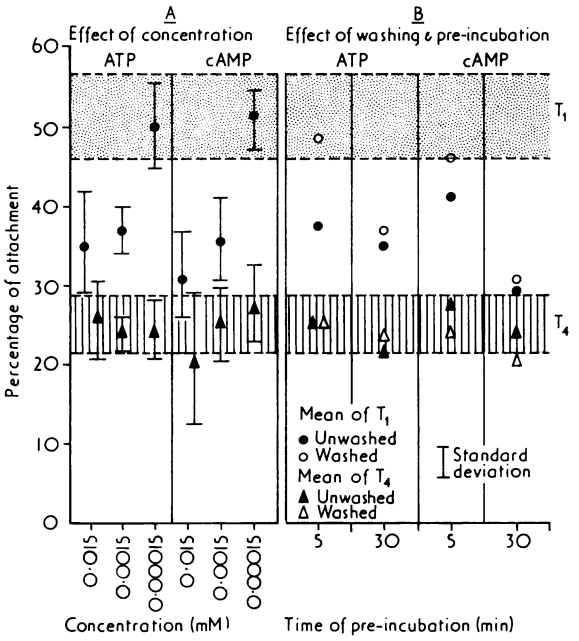

FIG. 3 *Effect of nucleotides, ATP and cAMP, on ability of sperm to participate in attachment by untreated gonococci. Sperm were pre-incubated for* 30 *min. at* 35°*C with the nucleotide before mixture with T1 or T4 bacteria. After the mixtures had been incubated for an additional* 15 *min. in a water bath shaker at* 35°*C, the percentage of sperm with gonococci attached was determined. Various concentrations of ATP and cAMP were used in experiments shown in A. In B, sperm were pre-incubated with* 0·015 *mM ATP or cAMP, and then, in one experiment (open circles or squares), sperm were washed twice by centrifugation before mixture with T1 or T4 gonococci. Sperm were not washed after pre-incubation in the other experiment (closed circles or squares)*
Stippled and lined areas show expected range for percentage of attachment of T1 and T4 gonococci respectively, when bacteria and sperm were incubated under standard conditions for 15 *min. (see Fig.* 1).

Lentner, 1970). Removal of calcium from the RS did not affect attachment.

Effect of nucleotides

The nucleotides, ATP and cAMP, affected sperm but not bacteria. Sperm pre-incubated for 30 min. with 0·015 to 0·0015 mM of either nucleotide (Fig. 3A) had a significantly lower percentage of attachment than either controls or those sperm treated with 0·00015 mM of the compounds. Nucleotides (0·015 mM) interfered with attachment of T1 gonococci after 5 min. of pre-incubation (Fig. 3B). This effect was removed if sperm were washed before mixture with bacteria, but after 30 min. pre-incubation, the interference was not removed.

Effect of enzymes

Pre-incubation of sperm with 10 mg./ml. of trypsin removed and degraded most sperm tails; however, lesser concentrations of this enzyme did not damage sperm nor did they affect attachment of gonococci to the treated sperm. Lysozyme and alpha chymotrypsin at concentrations of 10 mg./ml. or less neither damaged sperm nor interfered with attachment of gonococci to treated sperm.

Pre-incubation of pilated T1 gonococci with any of three enzymes, trypsin, alpha chymotrypsin, and lysozyme, did interfere with the ability of the

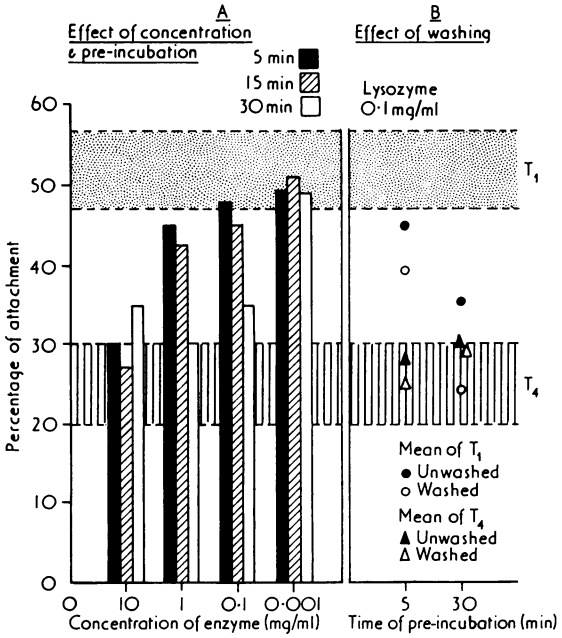

FIG. 4 *Effect of lysozyme on ability of T1 and T4 gonococci to attach to untreated sperm. T1 gonococci were pre-incubated at 35°C for various lengths of time with different concentrations of lysozyme before mixture of bacteria with sperm (A). After the mixtures had been incubated for 15 min. in a water bath shaker at 35°C, the percentage of sperm with gonococci attached was measured. In B, gonococci pre-incubated with 0·1 mg./ml. lysozyme for 5 or 30 min. were washed twice by centrifugation (open circles and squares) or not washed (closed circles and squares) before mixture with sperm. These mixtures were incubated for an additional 15 min. in a water bath shaker at 35°C, and then the percentage of sperm with gonococci attached was determined. Stippled and lined areas show expected range for percentage of attachment of T1 and T4 gonococci respectively, when bacteria and sperm were incubated under standard conditions for 15 min. (see Fig. 1).*

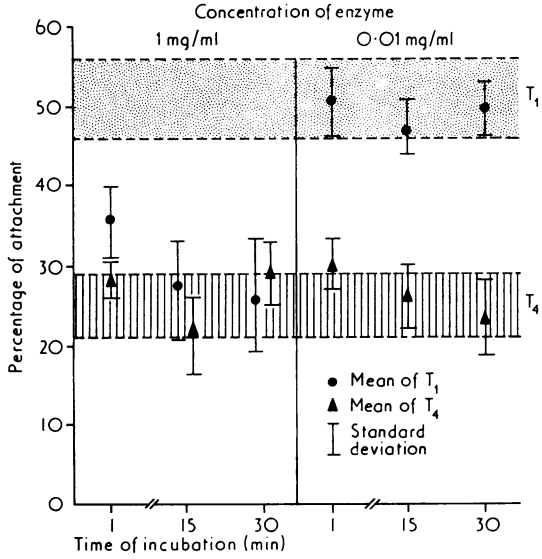

FIG. 5 *Effect of 1·0 or 0·01 mg./ml. trypsin on percentage of attachment to sperm by T1 or T4 gonococci. Bacteria were pre-incubated for 30 min. at 35°C with the enzyme and then mixed with sperm. Percentage of sperm with bacteria attached was determined after 1, 15, or 30 min. incubation at 35°C in a water bath shaker.*
Stippled and lined areas show expected range for percentage of attachment of T1 and T4 gonococci respectively, when bacteria and sperm were incubated under standard conditions for 15 min. (see Fig. 1).

bacteria to attach to the sperm. Both the concentration of enzyme and the time of pre-incubation influenced the reduction in percentage of attachment. It was found that 5 min. of pre-incubation of bacteria with 10 mg./ml. of lysozyme or 30 min. with 1·0 mg./ml. of the enzyme reduced attachment of T1 gonococci to the level expected for T4 bacteria (Fig. 4A). When gonococci were pre-incubated for 5 min. with 0·1 mg./ml. of lysozyme, the ability of T1 bacteria to attach was only slightly reduced (Fig. 4B). However, T1 gonococci washed after 30 min. pre-incubation attached to as few sperm as did T4 bacteria.

Pre-incubation of gonococci for 30 min. with 0·01 mg./ml. of trypsin resulted in normal attachment of T1 and T4 bacteria (Fig. 5). Identical treatment with 1·0 mg./ml. of trypsin produced T4 gonococci that attached as expected, and T1 gonococci that attached to the same percentage of sperm as non-pilated T4 bacteria. Electron microscopy of negatively stained gonococci after treatment with 1·0 mg./ml. of trypsin showed fewer pili (Fig. 6B) than bacteria not treated with the enzyme but incubated under the same conditions (Fig. 6A). T1 gonococci pre-incubated with this concentration of lysozyme and alpha chymotrypsin also were depilated. No morphological changes in T4 bacteria were seen. Neuraminidase had no effect on attachment when either the sperm, the bacteria, or the mixture was pre-incubated for 30 min. with as much as 0·5 ml. of the enzyme per 0·5 ml. of the sample.

Discussion

Gonococci can attach to red blood cells (Punsalang and Sawyer, 1973), to secretory cells lining the Fallopian tubes (Ward, Watt, and Robertson, 1974), to urethral mucosal cells (Ward, and Watt 1972), to

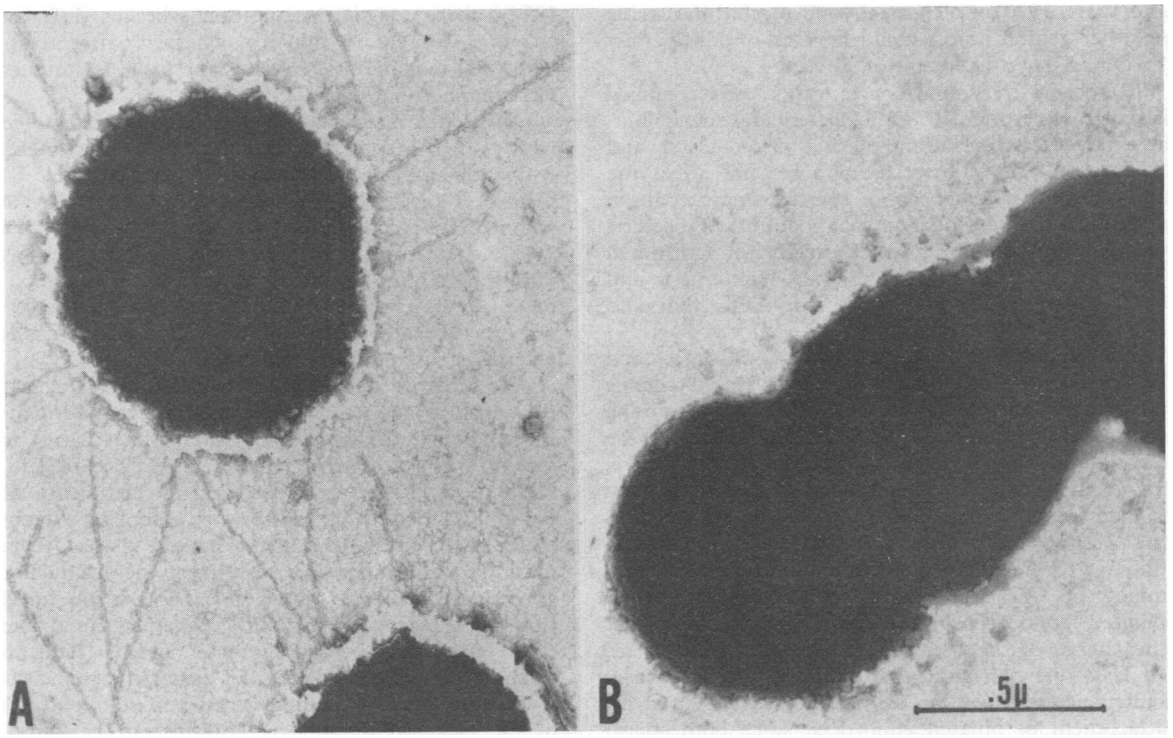

FIG. 6 *Electron micrograph of T1 gonococci treated and untreated with 1·0 mg./ml. trypsin. T1 gonococci were suspended in Ringer's solution, the suspension was divided into two portions of 0·9 ml. each, and 0·1 ml. trypsin solution (10 mg./ml.) was added to one tube and 0·1 ml. RS to the other. Both portions were incubated for 30 min. in a water bath shaker at 35°C, washed twice by centrifugation, negatively stained, and then examined by electron microscopy. Numerous pili are seen on untreated gonococci (A), while treated bacteria have none (B).* ×52,000.

leucocytes (Punsalang and Sawyer, 1973; Swanson, 1973; Swanson and others, 1974; Thomas, Hill, and Tyeryar, 1973), to epithelial cells (Punsalang and Sawyer, 1973), to cells in tissue culture (Swanson, 1973), and to human sperm (James-Holmquest and others, 1974). However indiscriminately these bacteria attach as a species, they are more selective when isolated by colony type and pilation. There are confusing reports on the interaction of gonococci with leucocytes (Ofek, Beachey, and Bisno, 1974; Punsalang and Sawyer, 1973; Swanson and others, 1974; Thomas and others, 1973; Thongthai and Sawyer, 1973), but pilated T1 gonococci demonstrated superior adherence to all other cell types examined.

In the model system described, phagocytosis of gonococci could not occur, so attachment could be studied as an isolated phenomenon. Both sperm and T1 gonococci apparently possessed different receptor sites on their surfaces for attachment. Various agents affected precentage of attachment by action either on bacteria or on sperm, but no agent affected both kinds of cells. These data suggest that attachment is more complex than a simple sticking together of the eukaryotic and prokaryotic cells.

The most dramatic factor that affected the bacteria was iron salt, which greatly enhanced attachment (Fig. 3). Gonococci treated with iron adhered to sperm so avidly that they appeared like iron filings attached to a magnet. Kellogg and others (1963) suggested that iron added to media enhanced differences between colonial types. Presence of iron did not increase pilation but could effect some other change in the cell wall, an hypothesis that is strengthened by the enhanced attachment of nonpilated T4 bacteria. Various bacterial infections are enhanced *in vivo* by the presence of iron (Weinberg, 1974), and 'nutritional' immunity has been proposed to explain the host's response to reduce the amount of iron available to invading pathogens. Augmented ability to attach to human cells would increase the probability of success of a gonococcal infection (Ward

and Watt, 1973). Calcium, if concentrated in uterine or other genital fluids, could have an opposite effect by depressing attachment of T1 cells.

Copper in very low concentration is recognized as both a spermicidal and a bactericidal metal, but, at a mortal concentration (Fiscina, Oster, Oster, and Swanson, 1973), cupric sulphate did not affect the ability of either gonococci or sperm to participate in attachment. The evidence that viability of bacteria was not required for normal attachment was noted previously in our system (James-Holmquest and others, 1974) and that of others (Thongthai and Sawyer, 1973).

The report of Mercado, Hicks, Orago, and Rosado (1974) that ATP and cAMP might change polarity of sperm membranes or alter membrane configuration suggested that treatment with these compounds might identify sperm as an active, involved participant or simply as a passive partner to attachment. The ability of nucleotides to alter the sperm to interfere with attachment of T1 bacteria but not to affect the gonococci indicated that there might be sites on sperm membranes to which piliated gonococci preferentially attached, or that the charge of the membrane facilitated attachment. At least one biochemical site on the sperm surface, sialic acid residues, can be ruled out as essential for attachment of piliated gonococci, since both sperm and bacteria treated with neuraminidase (Goodhart, 1969) attached as expected.

Certain cell wall features of T1 gonococci play a significant role in attachment. Trypsin, lysozyme, and alpha chymotrypsin, enzymes found in cervical mucus (Maghissi, 1969; Schumacher and Pearl, 1969; Shih, Kennedy, and Huggins, 1940) all reduced attachment by T1 gonococci, and the effect of each enzyme was enhanced when treated bacteria were washed by centrifugation. These data would be simpler to interpret if all three enzymes were proteolytic, or if lysozyme had not been effective. However, it may be speculated that some surface structures, most probably pili, are directly or indirectly affected by the enzymes, and further damage to these structures is incurred by the stress of centrifugation.

Pili are implicated as structures for attachment for several reasons. Pili have been reported to play a significant role in differential attachment because anti-pili antibody (James-Holmquest and others, 1974) blocked increased attachment by T1 bacteria. Few pili were detected after treatment of pilated T1 gonococci with 1·0 mg./ml. of a solution of purified crystalline trypsin in our studies (Fig. 6B) and those of Punsalang and Sawyer (1973). Such treated bacteria attached like nonpilated T4 gonococci. Since other investigators have reported normal pilation of T1 bacteria after treatment with a 0·25 per cent. trypsin solution (Swanson and others, 1974), there remains a question whether different enzymes do remove pili, or whether, after treatment, pili remain undetected by the usual techniques of preparation and staining.

Other factors in addition to pili and associated with both T1 and T4 gonococci affect attachment. Although enzyme treatment of nonpilated T4 gonococci of strains WP and MS11 and T4* of strain MS11 was without effect, the presence of iron salts did enhance attachment. Because of the short duration of these experiments, differences in attachment should not be attributed to metabolic processes, but are probably the results of alterations in the external structure of either bacteria or sperm.

The evidence that both the sperm and particularly the virulent gonococci have different requirements for attachment suggests that surface sites active in attachment are different for these two types of cells. If all these factors are considered in relation to the pathophysiology of gonorrhoea, an interesting hypothesis may be devised. In the natural disease, there may be substances like iron that help attached gonococci to remain on sperm for possible travel through the female and male urogenital tract, and there also may be physiological agents such as calcium or various enzymes that detach the gonococci within the uterus or Fallopian tube. The hypothesis of the bacterial hitchhiker (Howard, 1971; James-Holmquest and others, 1974) is enhanced by demonstration of the involvement of different surface structures of bacteria and of sperm in attachment and by evidence that physiological conditions do affect attachment.

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